Protein/DNA Arrays

(Spin Column Separation Version)

User Manual

Cat # MA1210, MA1211, MA1212

MA1213, MA1214 & MA1215

P/N 13299 Rev. B 032007

Panomics

Panomics, Inc.

Protein/DNA Array Kit User Manual

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If a paper cites the Protein/DNA Array kit and is published in a research journal, the lead author(s) may receive a travel stipend for use at a technology conference or tradeshow by sending a copy of the paper to our technical support group at techsupport@panomics.com or via fax at (510) 818-2610.

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About the User Manual

Who Should Read this Manual

Anyone that has purchased a Protein/DNA Array Kit from Panomics to profile from 56 to 345 different Transcription Factors.

What this Manual Covers

This manual provides recommendations and step-by-step procedures for the following:

- Sample and assay preparation
- Assay procedure
- Troubleshooting
- Layouts

Safety Warnings and Precautions

CAUTION All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.

For More Information

For information about the Protein/DNA products mentioned in this manual, visit our website at www.panomics.com.

About the Protein/DNA Assay Kit

Protein/DNA Assay

The Protein/DNA kit is a highthroughput profiling assay that has the ability to mesaure the activation of multiple TF's from a single sample .

The cytokine panels for TranSginal Protein/DNA kits are available as:\

Array Name	Cat. #	# of TF's
♦ PD Array I	MA1210	56
♦ PD Array II	MA1211	96
♦ PD Array III	MA1212	94
♦ PD Array IV	MA1213	76
♦ PD Array V	MA1214	73
♦ Combo Array	MA1215	345

Protein/DNA Assay Kit Contents and Handling Conditions

Kit Contents and Storage

The contains the following components. Shelf life of kit is six months from date of receipt under proper storage conditions

Box 1: Array Membranes and Hybridization Reagents (Store at 4 °C)

- Protein/DNA Array (3 each)
- Spin Column (3 each)
- Spin Column collection Tube (6 each)
- 1X Column Incubation Buffer (2 ml)
- 1X Colunm Wash Buffer (10 ml)
- Hybridization Buffer (15 ml)
- 20X SSC (32 ml)
- 20% SDS (15 ml)
- Streptavidin-HRP conjugate (60 µl)
- 4X Wash Buffer (45 ml)
- Solution I (750 µl)
- Solution II (750 µl)
- Solution III (8 ml)

Box 2: Reaction Kit (Store at -20 °C)

- Probe Mix (30 µl)
- 1X Column Elution Buffer (200 µl) Bring to room temperature before use
- Distilled H_20, RNAse, DNAse free (500 µl)
- Control Nuclear Extract from HeLa Cells (5 µl)
- 2X Blocking Buffer (30 ml)
- 1X Detection Buffer (60 ml)
- 5X Pre-Treatment Buffer I (20 ml)
- 5X Pre-Treatment Buffer II (20 ml)

Additional Materials Required

- Panomics Nuclear Extraction Kit (Cat# AY2002)
- Deionized H_20

Materials and Euipment

- 0.5 ml and 1.5ml microfuge tubes
- Pipetman and tips
- Microcentrifuge
- Hybridization oven and bottles
- Plastic containers (~4.5” x 3.5”; equivalent to the size of a 200 µl pipete tip box)
- Shaker
- Plastic sheet protectors
- Hyperfilm™ ECL™ (Amersham, Cat # RPN3114K)
- Chemiluminescent Imaging system, FluorChem™ from Alpha Innotech

Introduction

Eukaryotic gene expression is regulated by a group of proteins called Transcription Factors (TFs). By interacting with specific DNA-Binding elements present in the promoters of certain genes, TFs modulate the frequency of transcriptional initiation. The expression or activity of TFs may be regulated in a cell-type , tissue specific, or cell cycle-dependent manner. Regulation can also be mediated by interactions with other proteins. Through different combinations of these regulatory mechanisms, eukaryotes are able to elicit a myriad of different gene expression patterns. The key to a full understanding of how gene expression is regulated is the analysis of the biochemical activity of TFs.

Panomics’ Protein/DNA Arrays simplify the functional analysis of eukaryotic TFs. Our array-based technology is a significant improvement over cumbersome gel mobility-shift assays, which permit the characterization of only a single TF at a time. With these Protein/DNA Arrays, you can profile the activities of multiple TFs simultaneously. These arrays can be used to study TF activation in a variety of biological processes, including cell proliferation, differentiation, transformation, apoptosis, and drug treatment.

We currently offer six versions of the TranSignal Protein/DNA Array. Version I for 56 transcription factors (TFs); Version II for 96; Version III for 94; Version IV for 76; Version V for 73; and the Combo Array for 345 TFs. These transcription factors are well characterized and widely available in published literature.

Principicple of the Technology

Protein/DNA Arrays use a proprietary, patent-pending technology developed by Panomics for the high-throughput analysis of TF activation. The procedure is simple and straightforward (Figure 1). Three basic steps are involved: (1) a set of biotin-labeled DNA binding oligonucleotides (Probe Mix) are preincubated with a nuclear extract of interest to allow the formation of protein/DNA complexes; (2) the protein/DNA complexes are separated from the free probes; and (3) the probes in the complexes are then extracted and hybridized to the Protein/DNAl Array. Each kit includes the reagents for HRP-based chemiluminescence detection.

Overview of Assay Workflow

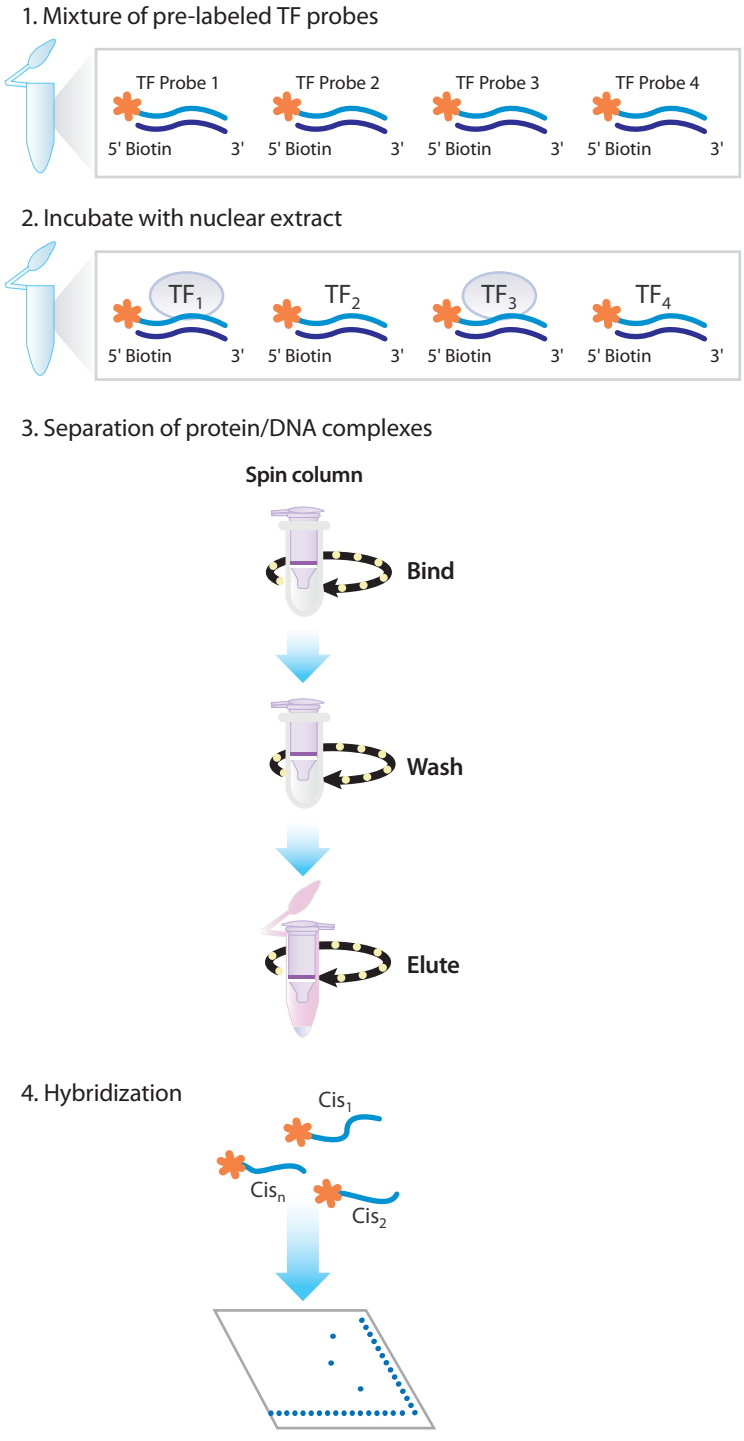

Figure 1: Workflow diagram of the Protein/DNA Array

Guidelines for Assay Design and Analysis

General Guidelines

- Read this user manual and all product inserts before performing the assay.
- Store all reagents at the recommended temperatures.
- Use Panomics' Nuclear Extraction Kit for best results.

Assay Procedures

Before You Start

- Thaw Positive Control and sample nuclear extracts on ice.
- Thaw Probe mix on ice.

Preparing Nuclear Extracts

- We recommend using Panomics' Nuclear Extraction Kit (Cat. #AY2002).
- The minimum protein concentration of nuclear extract should be 1.0 ug/ul or greater and we recommend using between 4–10 µg of Nuclear Extract Protein. You will need to adjust the amount of water accordingly when preparing the samples with the probe mix.
- When measuring protein concentration, we also recommend using the DC Protein assay from Bio-Rad (Lowry assay) with a plate reader or spectrophotometer.

Preparing the Membranes

In this Section, you will prepare the array membranes for use. If you are reusing membranes that have been previously stripped, skip steps , and begin from step 1.4.

1.1 Prepare Pre-Treatment Buffers I and II (see Appendix I for recipes) and warm the Hybridization Buffer to 42 °C in a water bath or incubator. If you notice a cloudy or soapy appearance for the Hybridization Buffer, make sure the particulates are completely dissolved before proceeding. It may require overnight heating in a water bath.

1.2 Place each array membrane into a hybridization bottle. Be sure to place the membrane in the hybridization bottle such that the spotted oligos face the center of the tube (away from the walls). Add 25 ml of 1X Pre-Treatment Buffer I to each bottle and place in a hybridization oven at 45 °C for 5 minutes.

1.3 Thoroughly decant the solution from each bottle. Add 25 ml of 1X Pre-Treatment Buffer II to each bottle and place in a hybridization oven at 45 °C for 10 minutes.

1.4 Decant the solution from each bottle. Rinse the entire surface of each membrane with distilled water (not provided), by filling each bottle approximately half full and swirling the bottle for 30 sec. Repeat this rinsing step two additional times and decant water after final rinse.

1.5 To each hybridization bottle, add 5 ml of prewarmed Hybridization Buffer (provided). Place each bottle into the rotating hybridization oven at 42 °C for 2 hr. If needed, you can stop at this point of the protocol and leave the membrane overnight at 42 °C.

Forming Protein-DNA Complexes

In this Section, you will allow the DNA probes to bind to transcription factors from your nuclear extract.

2.1 For each nuclear extract sample, combine the following components into a sterile 0.5-ml microcentrifuge tube (* = provided):

Nuclear extract (1-3 µg/µl)	5µl
TranSignal Probe Mix*	10µl
dH_2O (RNase,DNase free)*	5µl
Total Volume	20µl

Note If the protein concentration of your nuclear extract is below the recommended range, replace the dH_2O with additional nuclear extract.

2.2 Mix well by pipeting. Incubate samples for 30 min at 15 °C.

IsolatingTF-Bound Probes

In this section, you will isolate the protein-bound probes from the non-bound probes. All centrifuge steps should be carried out on a regular benchtop centrifuge, at 7,000 rpm at 4 °C, unless otherwise stated.

3.1 Wash Spin Column by adding 500 µl of chilled 1X Column Incubation Buffer and centrifuging at 10,000 rpm for 30 sec at 4 °C, or room temp.

3.2 Add 20 µl 1X Column Incubation Buffer to the TF-Probe mix, from Step 2.2. Transfer all of this mix into the center of the Spin Column.

3.3 Incubate the Spin Column on ice for 30 min.

3.4 Centrifuge column at 7,000 rpm for 30 sec at 4 °C and discard the flow through.

3.5 Place the Spin Column in a new Spin Column Collection Tube (provided).

3.6 Add 600µl of 1X Column Wash Buffer to the Spin Column and incubate for 10 min, on ice.

3.7 Centrifuge column at 7,000 rpm for 30 sec at 4 °C and discard the flow through.

3.8 Wash the column by adding 600 µl of 1X Column Wash Buffer to the Spin Column and centrifuging at 7,000rpm for 30 sec at 4 °C.

3.9 Repeat step 3.8 three more times.

3.10 Remove residual Wash Buffer by an additional centrifugation at 10,000 rpm for 30 sec at 4 °C

3.11 Add 60µl of 1X Column Elution Buffer to the center of the Spin Column and incubate at room temperature for 5 min.

3.12 Place the Spin Column in a clean 1.5 ml microcentrifuge tube and centrifuge for 1 minute at 10,000rpm at room temperature.

3.13 Place the microcentrifuge tube, containing the collected flow through, which contains the eluted probe, on ice and use for further steps. If needed, you can stop at this point and store the eluted probe at 20 °C overnight.

Hybridization

In this Section, you will hybridize the eluted, labeled probe prepared in Section 3 to the array membrane prepared in Section 1.

4.1 Denature the eluted probe (from step 3.13) by heating it at 95 °C for 3 min and quickly chill in ice for 2 min. Add the eluted probe to each hybridization bottle and hybridize at 42 °C overnight in a rotating hybridization oven

4.2 Decant the hybridization mixture from each hybridization bottle from Step 4.1 and wash each membrane as follows (* = See Appendix I for recipes):

4.3 Add 50 ml of prewarmed Hybridization Wash I*, incubate at 42°C for 20 min in a rotating hybridization oven. Decant liquid and repeat wash.

4.4 Add 50 ml of prewarmed Hybridization Wash II*, incubate at 42 °C for 20 min in a rotating hybridization oven. Decant liquid and repeat wash.

Detection

Important: Do not allow the membrane to dry during the detection.

5.1 Using forceps, carefully remove each membrane from its hybridization bottle and transfer to a clean container containing 20 ml of 1X Blocking Buffer; each membrane needs its own container. (We use a container that is equivalent to the size of a 200-µl pipet-tip container, ~4.5" x 3.5".)

5.2 Block the membrane by incubating at room temperature with the 1X Blocking Buffer for 15 min with gentle shaking.

5.3 Remove 1 ml of the 1X blocking buffer to a clean microcentrifuge tube. Dilute 20 µl of the stock Streptavidin-HRP conjugate with 1 ml of the 1X Blocking Buffer. Vortex the diluted streptavidin-HRP and transfer back into the 1X Blocking Buffer from Step 5.2 (containing the membrane). Add the diluted conjugate to the container, making sure not to pour directly on the membrane. Continue shaking the membrane for 15 min at room temperature.

5.4 Decant the diluted Streptavidin-HRP solution. Wash each membrane three times at room temperature with 20 ml of 1X Wash Buffer, each for 8 min.

5.5 Add 20 ml of 1X Detection Buffer to each membrane and incubate at room temperature for 5 min.

5.6 Overlay each blot with 2.5 ml of Working Substrate Solution, prepared by mixing the following, in order: 250 µl Solution I with 250 µl Solution II. Briefly vortex and add 2.0 ml Solution III. Thoroughly mix. Using a plastic sheet protector or overhead transparency film (do not use thin plastic cling wraps as these wrinkle and lead to uneven detection across the membrane). Place each membrane on a plastic sheet. Then, pipet 2.5 ml of the mixed substrate solution onto each membrane and overlay each with a second plastic sheet on a flat surface as quick as possible. Ensure that substrate is evenly distributed over the membrane with no air bubbles. Incubate at room temperature for 5 min.

5.7 Remove excess substrate by gently applying pressure over the top sheet. Using a paper towel, remove excess substrate that might be remaining on the surface of the sheets. Expose the membranes using either Hyperfilm ECL (2-10 min) or a chemiluminescence imaging system (5-15 min), such as the FluorChem imager from Alpha Innotech Corp. In either case, we recommend that you try several different exposure times.

5.8 Obtain quantitative analysis, if desired. If you are using a chemiluminescence imaging system, follow the instructions that are provided with that system's software. If you are using Hyperfilm ECL, you will need to scan the film to obtain numerical data for comparison.

Results & Analysis

TranSignal Arrays give you quick answers when you want to identify those activated transcription factors through comparison of two (or more) samples. Follow these guidelines to analyze your results:

6.1 Acquire the images using either x-ray film or a chemiluminescence imaging system.

6.2 Adjust the exposure time such that the majority of the positve control spots have equal signal intensity at the same exposure time.

6.3 When comparing mutliple blots, ensure that the control spots have a similar intensity.

Stripping Procedure

Note: We do not encourage stripping the array membranes more than two times.

7.1 Wash membranes in 0.4M NaOH at 45 °C for 30 min.

7.2 Wash membranes in 0.2M Tris-HCL, pH 7.6, 0.1X SSC, and 0.1% SDS at 45 °C for 15 min.

7.3 To ensure that stripping was successful, follow the standard chemiluminescence detection procedure as described in the user manual.

7.4 After detection, wash the membrane twice in 1X TBST with 0.1%SDS at 42 °C for 30 min.

7.5 Membranes are ready for hybridization or air dry the membrane at room

7.6 Store dry membrane protected between paper at room temperature unil ready for further use.

Recipe for 10X TBST

1M Tris-HCL pH 7.5, 1.5M NaCl, 0.5% Tween-20

Possible Problems and Recommended Solutions

Observation	Possible Cause	Recommended Action
Uneven Background	Substrate is note evenly distributed on the membrane	Do not use thin cling wrap materials during the overlay procedure
	Exposure Time to Short	Re-detect with substrate that is evenly covering the membrane surface
High background	Incubation with substrate is too long	Incubate with shorter time
	Non-specific protein bound to membrane	Increase blocking or washing times.
Signal is too weak	Not enough protein was used in the assay.	Carefully measure the concentration of the nuclear extract using a Lowry or Bradford assay

Observation	Possible Cause	Recommended Action
Don't see difference between treated and untreated samples	Confirm concentration of nuclear extract	Carefully measure concentration
	Cells cultured with growth factors in serum	Culture cells overnight without serum prior to treatment.
	TF not activated	Confirm with EMSA

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APPENDIX I

RECIPES AND INSTRUCTIONS

•75 ml of 1X Pre-Treatment Buffer I

Warm 5X Pre-Treatment Buffer I to room temperature. If precipitate is visible, warm gently to fully dissolve solution, and allow to cool to room temperature. To 60 mL of deionized H_2O, add 15 ml of 5x Pre-Treatment Buffer I. Mix well.

•75 ml of 1X Pre-Treatment Buffer II

Warm 5X Pre-Treatment Buffer II to room temperature. If precipitate is visible, warm gently to fully dissolve solution, and allow to cool to room temperature. To 60 mL of deionized H_2O, add 15 ml of 5x Pre-Treatment Buffer II. Mix well.

•300 ml of 2X SSC/0.5% SDS (Hybridization Wash I)

To 262.5 ml of deionized H2O, add 30 ml of 20X SSC (provided) and 7.5 ml of 20% SDS (provided). Mix well.

•300 ml of 0.1X SSC/0.5% SDS (Hybridization Wash II)

To 291 ml of deionized H2O, add 1.5 ml of 20X SSC (provided) and 7.5 ml of 20% SDS (provided). Mix well.

•60 ml of 1X Blocking Buffer

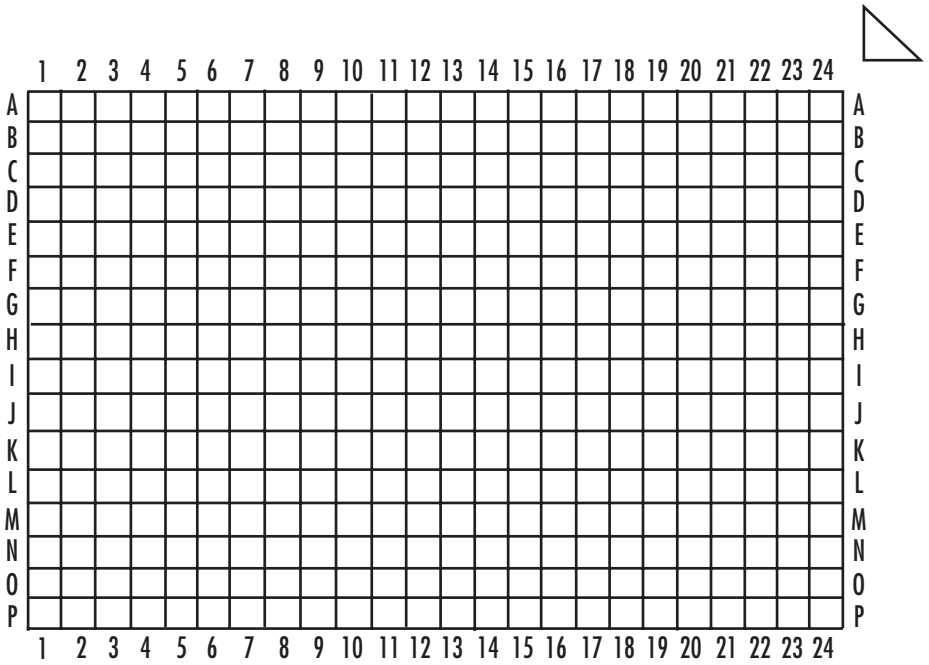
To 30 ml of deionized H2O, add 30 ml of 2X Blocking Buffer (provided). Mix well and store at 4°C.

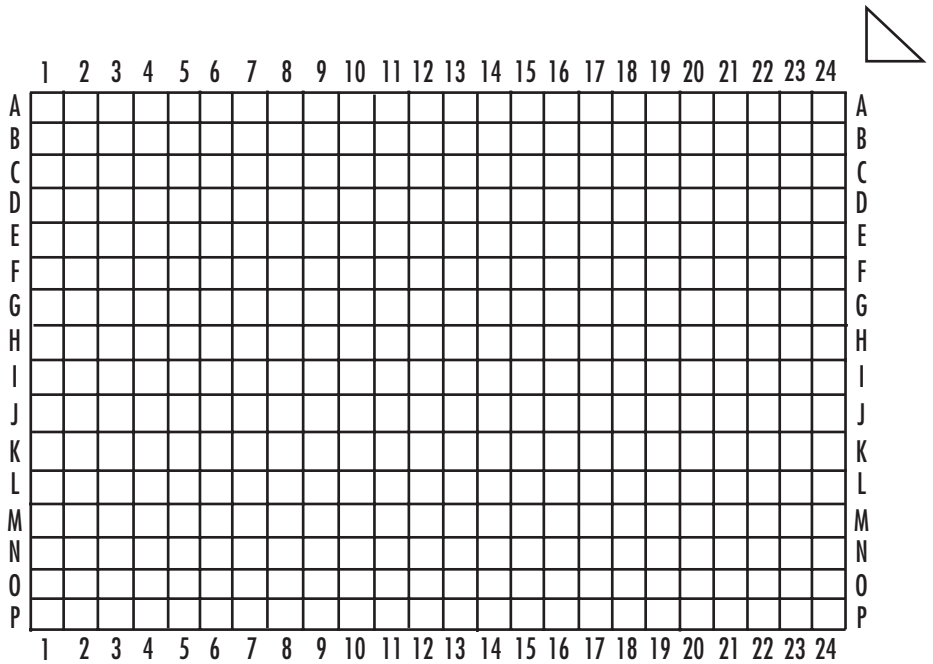
•180 ml of 1X Wash Buffer

To 135 ml of deionized H2O, add 45 ml of 4X Wash Buffer (provided). Mix well and store at room temperature.

TranSignal Protein/DNA Array Grid

Photocopy this page onto a separate piece of paper or transparency film. Note that the notch is on the upper-right hand corner of the grid.





Array I: Schematic Diagram of TranSignal™ Protein/DNA Array I

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
A	AP-1 (1)	AP-1 (1)	AP-2 (1)	AP-2 (1)	AR	AR	Brn-3	Brn-3	CEBP (1)	CEBP (1)	CBF	CBF	CDP	CDP	c-Myb	c-Myb	AP-1 (2)	AP-1 (2)	A
B	AP-1 (1)	AP-1 (1)	AP-2 (1)	AP-2 (1)	AR	AR	Brn-3	Brn-3	CEBP (1)	CEBP (1)	CBF	CBF	CDP	CDP	c-Myb	c-Myb	AP-1 (2)	AP-1 (2)	B
C	CREB (1)	CREB (1)	E2F-1	E2F-1	EGR (1)	EGR (1)	ER	ER	Ets (1)	Ets (1)	Ets-1/Pea-3 (1)	Ets-1/Pea-3 (1)	FAST-1	FAST-1	GAS/ISRE	GAS/ISRE	AP-2 (2)	AP-2 (2)	C
D	CREB (1)	CREB (1)	E2F-1	E2F-1	EGR (1)	EGR (1)	ER	ER	Ets (1)	Ets (1)	Ets1/Pea3 (1)	Ets1/Pea3 (1)	FAST-1	FAST-1	GAS/ISRE	GAS/ISRE	AP-2 (2)	AP-2 (2)	D
E	GATA	GATA	GR/PR	GR/PR	HNF-4 (1)	HNF-4 (1)	IRF-1	IRF-1	MEF-1 (1)	MEF-1 (1)	MEF-2 (1)	MEF-2 (1)	Myc/Max	Myc/Max	NF-1 (1)	NF-1 (1)			E
F	GATA	GATA	GR/PR	GR/PR	HNF-4 (1)	HNF-4 (1)	IRF-1	IRF-1	MEF-1 (1)	MEF-1 (1)	MEF-2 (1)	MEF-2 (1)	Myc/Max	Myc/Max	NF-1 (1)	NF-1 (1)			F
G	NFAT-1 (1)	NFAT-1 (1)	NF-E1/YY1	NF-E1/YY1	NF-E2 (1)	NF-E2 (1)	NFkB (1)	NFkB (1)	OCT (1)	OCT (1)	p53 (1)	p53 (1)	Pax-5 (1)	Pax-5 (1)	Pbx-1	Pbx-1			G
H	NFAT-1 (1)	NFAT-1 (1)	NF-E1/YY1	NF-E1/YY1	NF-E2 (1)	NF-E2 (1)	NFkB (1)	NFkB (1)	OCT (1)	OCT (1)	p53 (1)	p53 (1)	Pax-5 (1)	Pax-5 (1)	Pbx-1	Pbx-1			H
I	Pit-1 (1)	Pit-1 (1)	PPAR (1)	PPAR (1)	PRE	PRE	RAR/DR-5	RAR/DR-5	RXR/DR-1	RXR/DR-1	SIE	SIE	Smad/SBE	Smad/SBE	SMAD-3/4	SMAD-3/4			I
J	Pit-1 (1)	Pit-1 (1)	PPAR (1)	PPAR (1)	PRE	PRE	RAR/DR-5	RAR/DR-5	RXR/DR-1	RXR/DR-1	SIE	SIE	Smad/SBE	Smad/SBE	SMAD-3/4	SMAD-3/4			J
K	Sp-1	Sp-1	SRE	SRE	Stat-1	Stat-1	Stat-3	Stat-3	Stat-4	Stat-4	Stat-5	Stat-5	Stat-6	Stat-6	TFIID	TFIID			K
L	Sp-1	Sp-1	SRE	SRE	Stat-1	Stat-1	Stat-3	Stat-3	Stat-4	Stat-4	Stat-5	Stat-5	Stat-6	Stat-6	TFIID	TFIID			L
M	TR	TR	TR/DR-4	TR/DR-4	USF-1 (1)	USF-1 (1)	VDR/DR-3	VDR/DR-3	HSE	HSE	MRE	MRE							M
N	TR	TR	TR/DR-4	TR/DR-4	USF-1 (1)	USF-1 (1)	VDR/DR-3	VDR/DR-3	HSE	HSE	MRE	MRE							N
O																			O
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		

Array V: Grey rows and columns are spotted with biotinylated DNA and are used as postive controls for the assay. Pink shaded Rows are the same TF's as the TF's above in the white rows except that they are spotted at a 1:10 dilution. For some Transcription Factors, mutliple consensus sequences for the same TF have been publsihed in the literature and where appropriate, we have provided those variations on our arrays. For the TF's with mutliple consensus sequences, they have been denoted with numbers in parenthesis, ie (1), (2), (3) etc. To obtain the consensus sequences and references for the TF's, used in our arrays, please call our Technical Support group at 877-726-6642 or email at techsupport@panomics.com.

Array II: Schematic Diagram of TranSignal™ Protein/DNA Array II

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	ADR-1	ADR-1	AHR/ARNT	AHR/ARNT	ANG-IRE	ANG-IRE	Antioxident RE	Antioxident RE	AP-3 (1)	AP-3 (1)	AP-4 (1)	AP-4 (1)	β-response element	β-response element	CdxA/ NKX2	CdxA/ NKX2	
B	CEF-1 (1)	CEF-1 (1)	CEF-2 (1)	CEF-2 (1)	CETP/CRE	CETP/CRE	COUP-TF (1)	COUP-TF (1)	CREB-BP1	CREB-BP1	c-REL	c-REL	DR-0	DR-0	DR-1	DR-1	
C	DR-2	DR-2	DR-3	DR-3	DR-4	DR-4	DR-5	DR-5	E47	E47	E4BP-4 (1)	E4BP4 (1)	E4F/ATF	E4F/ATF	Elk-1	Elk-1	
D	EVI-1	EVI-1	FKHR (1)	FKHR (1)	FKHR (2)	FKHR (2)	Afxh/ Foxo4	Afxh/ Foxo4	Freac-2 (1)	Freac-2 (1)	Freac-2 (2)	Freac-2 (2)	Freac-4	Freac-4	Freak-7	Freak-7	
E	GAG	GAG	GAS	GAS	GATA-1 (1)	GATA-1 (1)	GATA-1/2	GATA-1/2	GATA-2	GATA-2	GATA-3	GATA-3	GATA-4	GATA-4	GATA-6	GATA-6	
F	Gfi-1	Gfi-1	HAS	HAS	HBS (1)	HBS (1)	HBS/HAS	HBS/HAS	HFH-1 (1)	HFH-1 (1)	HFH-2	HFH-2	HFH-3 (1)	HFH-3 (1)	HFH-8	HFH-8	
G	HIF-1	HIF-1	HNF-3	HNF-3	HNF-3β	HNF-3β	HNF-4	HNF-4	HNF-4/ COUP-TF	HNF-4/ COUP-TF	Ikaros	Ikaros	IRF-1/2	IRF-1/2	ISRE (1)	ISRE (1)	
H	ISRE (2)	ISRE (2)	ISRE (3)	ISRE (3)	L-III BP	L-III BP	KKLF	KKLF	MEF2 (1)	MEF2 (1)	MEF2 (2)	MEF2 (2)	MEF-3	MEF-3	MSP-1	MSP-1	
I	MT-Box	MT-Box	MUSF-1	MUSF-1	MZF-1	MZF-1	NF-Y	NF-Y	Nkx-2.5	Nkx-2.5	NPAS-2	NPAS-2	HBS/ XBP-1	HBS/ XBP-1	NZF-3	NZF-3	
J	ORE	ORE	p300	p300	PARP	PARP	PAX-2 (1)	PAX-2 (1)	PAX-3	PAX-3	PAX-4	PAX-4	PAX-6 (1)	PAX-6 (1)	PAX-8	PAX-8	
K	PPAR (2)	PPAR (2)	PPAR (3)	PPAR (3)	RREB (1)	RREB (1)	RREB (2)	RREB (2)	RSRFC4	RSRFC4	SAA	SAA	Skn	Skn	SRY (1)	SRY (1)	
L	Tax/CREB	Tax/CREB	TCF/LEF	TCF/LEF	XBP-1	XBP-1	XRE (1)	XRE (1)	ZIA	ZIA	ZIB	ZIB	ZIC	ZIC	ZID	ZID	
M																	

Array II: Grey rows and columns are spotted with biotinylated DNA and are used as postive controls for the assay. For some Transcription Factors, mutliple consensus sequences for the same TF have been publsihed in the literature and where appropriate, we have provided those variations on our arrays. For the TF's with mutliple consensus sequences, they have been denoted with numbers in parenthesis, ie (1), (2), (3) etc. To obtain the consensus sequences and references for the TF's, used in our arrays, please call our Technical Support group at 877-726-6642 or email at techsupport@panomics.com.

Array III: Schematic Diagram of TranSignal™ Protein/DNA Array III

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	ADD-1	ADD-1	AF-1/ ARP-1	AF1/ ARP-1	AFP-1	AFP-1	AIC/CBF	AIC/CBF	AML-1	AML-1	AP-2/YY1	AP-2/YY1	AREB-6	AREB-6	ARP	ARP	
B	ATF/CRE (1)	ATF/CRE (1)	ATF (2)	ATF (2)	CACC	CACC	CCAAT	CCAAT	CCAC	CCAC	CD28RC (1)	CD28RC (1)	CD28RC (2)	CD28RC (2)	Cdx-2	Cdx-2	
C	CEA	CEA	CP1/CTF/ CBTF	CP1/CTF/ CBTF	CPE	CPE	CREB-2	CREB-2	CSBP	CSBP	CTCF	CTCF	E12 (1)	E12 (1)	EBP-40/45	EBP-40/45	
D	EKLF (1)	EKLF (1)	EKLF (2)	EKLF (2)	ELF	ELF	H4TF	H4TF	HEN-1	HEN-1	HFH-8/ HNF3 LUN	HFH-8/ HNF3 LUN	HiNF	HiNF	HLF	HLF	
E	HMG	HMG	HNF-1A	HNF-1A	HOX4C	HOX4C	HOXD 8/9/10	HOXD 8/9/10	HOXD8 (1)	HOXD8 (1)	HOXD8 (2)	HOXD8 (2)	HOXD-9/10	HOXD-9/10	ICSBP	ICSBP	
F	Isl-1	Isl-1	KPF-1	KPF-1	KTP-1	KTP-1	LCR-F1	LCR-F1	LF-A1	LF-A1	LR-1	LR-1	LyF (1)	LyF (1)	LyF (2)	LyF (2)	
G	LyF-1 (3)	LyF-1 (3)	MAZ (2)	MAZ (2)	MTB-Zf	MTB-Zf	MTF (1)	MTF (1)	MyoD	MyoD	MyoG	MyoG	MyTI	MyTI	MZF-1 (1)	MZF-1 (1)	
H	MZF-1 (2)	MZF-1 (2)	NF-4FA	NF-4FA	NF-Gma (1)	NF-Gma (1)	NF-Gma (2)	NF-Gma (2)	NF-IL-2	NF-IL-2	NF-Y (2)	NF-Y (2)	NRF-1	NRF-1	P53 (3)	P53 (3)	
I	p55	p55	PEA3/HIP	PEA3/HIP	PEBP	PEBP	PEBP-2 (1)	PEBP-2 (1)	PPUR (1)	PPUR (1)	PPUR (2)	PPUR (2)	Pur-1	Pur-1	PYR	PYR	
J	RB	RB	RFX-1/2/3 (1)	RFX-1/2/3 (1)	RIPE-3a1	RIPE-3a1	SIF-1	SIF-1	SIF-2	SIF-2	SIF-3	SIF-3	SP-1/ASP	SP-1/ASP	SRF/SAP	SRF/SAP	
K	SYR (2)	SYR (2)	TCE	TCE	TFE-3	TFE-3	Tf-LF	Tf-LF	TGT-3	TGT-3	TIF-1	TIF-1	TREF-1/2	TREF-1/2	v-Maf	v-Maf	
L	WTI (1)	WTI (1)	WTI (2)	WTI (2)	WTI (3)	WTI (3)	X2 BP	X2 BP	XBP-1/ X2 BP	XBP-1/ X2 BP	ZNF174	ZNF174					
M																	

Array III: Grey rows and columns are spotted with biotinylated DNA and are used as postive controls for the assay. For some Transcription Factors, mutliple consensus sequences for the same TF have been publsihed in the literature and where appropriate, we have provided those variations on our arrays. For the TF's with mutliple consensus sequences, they have been denoted with numbers in parenthesis, ie (1), (2), (3) etc. To obtain the consensus sequences and references for the TF's, used in our arrays, please call our Technical Support group at 877-726-6642 or email at techsupport@panomics.com.

Array IV: Schematic Diagram of TranSignal™ Protein/DNA Array IV

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
A	AAF	AAF	ABF-1	ABF-1	ACF	ACF	ACP BP	ACP BP	AIC-2/3/4	AIC-2/3/4	ALF-1	ALF-1	α-PAL	α-PAL		A
B	AP-2 (2)	AP-2 (2)	AP-3 (2)	AP-3 (2)	AP-4 (2)	AP-4 (2)	apo A1 PR	apo A1 PR	ATF-adelta	ATF-adelta	ATF-α	ATF-α	βM-globin Factor B1	βM-globin Factor B1		B
C	BZP	BZP	CEBP (2)	CEBP (2)	CEPB (3)	CEPB (3)	CEBP (4)	CEBP (4)	CEBP (5)	CEBP (5)	CBFB	CBFB	CBF (2)	CBF (2)		C
D	c-Ets-1	c-Ets-1	c-fos enhancerBP	c-fos enhancerBP	c-myb (2)	c-myb (2)	c-Myc	c-Myc	COUP-TF (2)	COUP-TF (2)	CP-1B	CP-1B	CP-1	CP-1		D
E	CREB (2)	CREB (2)	CYP1A1	CYP1A1	DE-1	DE-1	dioxin receptor	dioxin receptor	E12/E47	E12/E47	E2F-1 (2)	E2F-1 (2)	E2	E2		E
F	E4BP4 (2)	E4BP4 (2)	EBP-80	EBP-80	EBP-C2/NF muE3/YEB3	EBP-C2/NF muE3/YEB3	EGF BP	EGF BP	EGR-1 (2)	EGR-1 (2)	EGR-2 (2)	EGR-2 (2)	EIL-1/2/3	EIL-1/2/3		F
G	ETF	ETF	ETV4/ E1A-F	ETV4/ E1A-F	Fra-1/JUN	Fra-1/JUN	gastrin EGF RE	gastrin EGF RE	GATA-1 (2)	GATA-1 (2)	GBF-1/2/3 HY5	GBF-1/2/3 HY5	GKLF	GKLF		G
H	H4TF-1	H4TF-1	HFH-11β/ 11α	HFH-11β/ 11α	HFH-1 (2)	HFH-1 (2)	HiNF-A	HiNF-A	HiNF-B/ H1TF1	HiNF-B/ H1TF1	HiNF-D3	HiNF-D3	HNF-1 a/b/c	HNF-1 a/b/c		H
I	HNF-1	HNF-1	HNF-4α 2/1	HNF-4α 2/1	HOXA4	HOXA4	HSF (2) distal	HSF (2) distal	HSF (3) proximal	HSF (3) proximal	HSF/HTF	HSF/HTF	IFI-56KBP	IFI-56KBP		I
J	IL-6 RE-BP	IL-6 RE-BP	ISGF	ISGF	kBF-A	kBF-A	lactoferrin BP	lactoferrin BP	LF-A1 (2)	LF-A1 (2)	LF-A2	LF-A2	LF-B2	LF-B2		J
K	LH2/Lim1	LH2/Lim1	LSF	LSF	LXRE-1	LXRE-1	MASH-1	MASH-1	MBP-1 (1)	MBP-1 (1)	MBP-1 (2)	MBP-1 (2)				K
L																L
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	

Array IV: Grey rows and columns are spotted with biotinylated DNA and are used as postive controls for the assay. For some Transcription Factors, mutliple consensus sequences for the same TF have been publsihed in the literature and where appropriate, we have provided those variations on our arrays. For the TF's with mutliple consensus sequences, they have been denoted with numbers in parenthesis, ie (1), (2), (3) etc. To obtain the consensus sequences and references for the TF's, used in our arrays, please call our Technical Support group at 877-726-6642 or email at techsupport@panomics.com.

Array V: Schematic Diagram of TranSignal™ Protein/DNA Array V

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
A	mck PR E1	mck PR E1	MDBP (1)	MDBP (1)	MDBP (2)	MDBP (2)	MEF-2a	MEF-2a	Mfh-1	Mfh-1	MHC gene PR W Box	MHC gene PR W Box	msx-1/2/3	msx-1/2/3		A
B	Myb (2)	Myb (2)	myc/CF1	myc/CF1	myc/PRF	myc/PRF	NCAM BP	NCAM BP	NF-1 (2)	NF-1 (2)	NF-1L	NF-1L	NF-A3	NF-A3		B
C	NF-A	NF-A	NFAT-1 (2)	NFAT-1 (2)	NF-Atp	NF-Atp	NF-Atx	NF-Atx	NF-E2 (2)	NF-E2 (2)	NFE-6/ CP-1	NFE-6/ CP-1	NFkB (2)	NFkB (2)		C
D	NFkB (3)	NFkB (3)	N-ras BP	N-ras BP	OCT (2)	OCT (2)	OCT (3)	OCT (3)	ODC	ODC	p53 (2)	p53 (2)	PAX1	PAX1		D
E	PAX-2 (2)	PAX-2 (2)	PAX-5 (2)	PAX-5 (2)	PAX-6	PAX-6	PBGD BP	PBGD BP	PCF	PCF	PEBP-2 (2)	PEBP-2 (2)	PEPCK PR GR	PEPCK PR GR		E
F	Pit-1 (1)	Pit-1 (1)	Pit-1 (2)	Pit-1 (2)	PO-B	PO-B	PRDI-BFc	PRDI-BFc	PRDII-BF1	PRDII-BF1	PREB	PREB	PTF-1β	PTF-1β		F
G	PTF-1	PTF-1	PU.1	PU.1	PUR	PUR	RAR/RXR CF1/MB67	RAR/RXR CF1/MB67	RFX-1/2/3	RFX-1/2/3	RORE	RORE	RVF	RVF		G
H	SAP-1b	SAP-1b	Snail	Snail	SPERM-1	SPERM-1	SRF (2)	SRF (2)	SRF (1)	SRF (1)	SSAP	SSAP	Stat-1/3	Stat-1/3		H
I	Stat-5b	Stat-5b	Surf-2 (2)	Surf-2 (2)	T3R	T3R	Tat	Tat	TFE-3L	TFE-3L	TFEB	TFEB	Thy-1 BP	Thy-1 BP		I
J	tPA BP	tPA BP	transferrin BP	transferrin BP	TTF-1 (1)	TTF-1 (1)	TTF-1 (2)	TTF-1 (2)	TxREF NF III	TxREF NF III	URE	URE	WAP BP	WAP BP		J
K	XBP-1 (2)	XBP-1 (2)	XRE (2)	XRE (2)	YB-1	YB-1										K
L																L
	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17

Array V: Grey rows and columns are spotted with biotinylated DNA and are used as postive controls for the assay. For some Transcription Factors, mutliple consensus sequences for the same TF have been publsihed in the literature and where appropriate, we have provided those variations on our arrays. For the TF's with mutliple consensus sequences, they have been denoted with numbers in parenthesis, ie (1), (2), (3) etc. To obtain the consensus sequences and references for the TF's, used in our arrays, please call our Technical Support group at 877-726-6642 or email at techsupport@panomics.com. PR= Promoter, BP= Binding Protein.

ComboArray: Schematic Diagram of Protein/DNA ComboArray

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	AP-1 (1)	GAS/ ISRE	Pbx1	Stat 6	CdxA/ NKX2	Freac-2 (1)	HFH-3	MT-Box	PPAR (2)	AML-1	CSBP	HOXD-8 (1)	MTF (1)	PPUR (1)	TGT-3	AP-2 (2)	c-myb BP	ETF	ISGF	MHC W box	NFkB (3)	PREB	T3R	
B	AP-2 (1)	GATA	Pit-1 (1)	TFIID	CEF-1 (1)	Freac-2 (2)	HFH-8	MUSF-1	PPAR (3)	AREB-6	CTCF	HOXD-8 (2)	MyoD	PPUR (2)	TIF-1	alpha-PAL	c-Myc	Fra-1/ JUN	kBF-A	msx-1/2/3	N-ras BP	PTF-1β	Tat	
C	AR	GR/PR	PPAR (1)	TR	CEF-2 (1)	Freac-4	HIF-1	MZF-1	RREB (1)	ARP	E12 (1)	HOXD-9/10	MyoG	Pur-1	TREF-1/2	AP-3 (2)	COUP-TF (2)	GATA-1 (2)	Lacto-ferrin BP	Myb (2)	OCT (3)	PTF-1	TEF-1/ AP-5	
D	Bm-3	IRF-1	PRE	TR/ DR-4	CETP-CRE	Freac-7	HNF-3 (1)	NF-Y (1)	RSRFC4	ATF/CRE	EBP-40/45	ICSBP	MyTI	PYR	v-Maf	ATF adelta	CP-1B	GBF-1/2 /3/HY5	LF-A1 (2)	myc/CF1	ODC	PU.1	TEF1	
E	CEBP (1)	MEF-1 (1)	RAR/ DR-5	USF-1 (1)	COUP-TF (1)	GAG	HNF-3 (2)	Nkx-2.5	SAA	ATF (2)	EKLF (1)	Isl-1	MZF1 (1)	RB	WTI (1)	ATF-a	CP-1	GKLF	LF-A2	myc/PRF	p53 (2)	PUR	TFE-3L	
F	CBF (1)	MEF-2 (1)	RXR/ DR-1	VDR/ DR-3	CREB-BP-1	GAS	HNF-4 (2)	NPAS-2	Skn	CACC	EKLF (2)	KPF-1	MZF1 (2)	RFX-1/ 2/3 (2)	WTI (2)	β M glob factor B1	CRE (2)	H4TF-1	LF-B2	NCAM BP	PAX-1	RFX-1/ 2/3 (3)	TFEB	
G	CDP	Myc-Max	SIE	HSE	c-Rel	GATA-1 (1)	HNF-4/ COUP-TF	NZF-3	SRY (1)	CCAAT	ELF	KTP-1	NF-4FA	RIPE3a1	WTI (3)	BZP	CYP1A1	HFH-11B/11a	LH2/ Lim-1	NF-1 (2)	PAX-2 (2)	RORE	Thy-1BP	
H	c-Myb (1)	NF-1 (1)	SMAD/ SBE	MRE	E47	GATA-1/2	Ikaros	ORE	Tax/ CREB	CCAC	HEN-1	LCR-F1	NF-Gma (1)	SIF-1	X2 BP	CEBP (2)	DE-1	HFH-1 (2)	LSF	NF-1/L	PAX-5 (2)	RVF	Trans-ferrin BP	
I	CREB (1)	NFAT-1 (1)	SMAD-3/4	ADR-1	E4BP-4 (1)	GATA-2	IRF-I/2	p300	TCF/LEF	CD28RC (1)	HFH-8/3 /LUN	LF-A1	NFIL-2	SIF-2	XBP-1 X2 BP	CEBP (3)	E12/E47	HiNF-A	LXRE-1	NF-A3	PAX-6 (2)	Snail	TTF-1 (1)	
J	E2F-1 (1)	NF-E1/ YY1	Sp-1	AhR/Amt	E4F/ATF	GATA-3	ISRE (1)	PARP	XBP-1	CD28RC (2)	HiNF	LR-1	NF-Y (2)	SIF-3	ZNF174	CEBP (4)	E2	HiNF-B/ HITF-1	MBP-1 (1)	NFAT-1 (2)	PBGD BP	SPERM-1	TxREF NF-III	
K	EGR (1)	NF-E2 (1)	SRE	ANG-IRE	Elk-1	GATA-4	ISRE (2)	PAX-2 (1)	XRE (1)	Cdx-2	HLF	LyF (1)	NRF-1	SP-1/ ASP	AAF	CEBP (5)	EBP-80	HiNF/D3	MBP-1 (2)	NF-Atp	PCF	SRF (2)	URE	
L	ERE	NFkB (1)	Stat-1	Antiox-ident RE	EVI-1	GATA-6	L-III BP	PAX-3	ADD-1	CEA	HMG	LyF (2)	p53 (3)	TCE	ABF-1	CBFB	EGF BP	HFN-1 a/b/c	MDBP (1)	NF-Atx	PEPCK PR	SSAP	v-rel 50-55k	
M	Ets (1)	OCT (1)	Stat-3	AP-3 (1)	FKHR (1)	GFI-1	KKLF	PAX-4	AF-1	CP1/CTF /CBTF	HNF-1A	LyF (3)	p55	SRF SAP	ACF	CBF (2)	EGR-1 (2)	HNF-4a 2/1	MDBP (2)	NF-E2 (2)	PO-B	Stat-1/3	WAP BP	
N	Ets/PEA3 (1)	p53 (1)	Stat-4	AP-4 (1)	FKHR (2)	HFH-1 (1)	MEF-3	PAX-6 (1)	AFP-1	CPE	HOX4C	MAZ	PEBP	TFE3	ACPBP	c-Ets-1	EGR-2 (1)	HOXA-4	MEF-2a	NF-E6/ CP1	PRDI-BFc	Stat-5b	XBP-1 (2)	
O	Fast-1	PAX-5 (1)	Stat-5	Beta-RE	Afxh/ Foxo4	HFH-2	MSP-1	PAX-8	AIC/CBF	CREB-2	HOXD-8/9/10	MTB-Zf	PEBP-2 (1)	Tf-LF	ALF-1	c-Fos BP	EIL-1/2/3	IL-6-RE-BP	Mfh-1	NFkB (2)	PRDII-BF1	Surf-2 (2)	YB-1	
P																								

ComboArray: Grey rows and columns are spotted with biotinylated DNA and are used as postive controls for the assay. For some Transcription Factors, mutliple consensus sequences for the same TF have been publsihed in the literature and whe appropriate, we have provided those variations on our arrays. For the TF's with mutliple consensus sequences, they have been denoted with numbers in parenthesis, ie (1), (2), (3) etc. To obtain the consensus sequences and references for the TF's, used in our arrays, please call our Technical Support group at 877-726-6642 or email at techsupport@panomics.com. RE= Respnose Element BP= Binding Protein